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Abstract: Recently, the neuropeptide S (NPS) neurotransmitter system has been identified as a promising psychopharmacological drug target given that NPS has shown anxiolytic-like and stress-reducing properties and memory-enhancing effects in rodent models. NPS binds to the G-protein-coupled receptor encoded by the neuropeptide S receptor gene (NPSR1). A functional variant within this gene leads to an amino-acid exchange (rs324981, Asn107Ile) resulting in a gain-of-function in the Ile107 variant which was recently associated with panic disorder in two independent studies. A potential psychopharmacological effect of NPS on schizophrenia psychopathology was demonstrated by showing that NPS can block NMDA antagonist-induced deficits in prepulse inhibition. We therefore explored a potential role of the NPSR1 Asn107Ile variation in schizophrenia. A case-control sample of 778 schizophrenia patients and 713 healthy control subjects was successfully genotyped for NPSR1 Asn107Ile. Verbal declarative memory and acoustic startle response were measured in subsamples of the schizophrenia patients. The case-control comparison revealed that the low-functioning NPSR1 Asn107 variant was significantly associated with schizophrenia (OR 1.19, $p=0.017$). Moreover, specifically decreased verbal memory consolidation was found in homozygous Asn107 carriers while memory acquisition was unaffected by NPSR1 genotype. The schizophrenia patients carrying the Ile107 variant demonstrated significantly reduced startle amplitudes but unaffected prepulse inhibition and habituation. The present study confirms findings from rodent models demonstrating an effect of NPS on memory consolidation and startle response in schizophrenia patients. Based on these findings, we consider NPS as a promising target for antipsychotic drug development.

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The functional coding variant Asn¹⁰⁷Ile of the neuropeptide S receptor gene (*NPSR1*) is associated with schizophrenia and modulates verbal memory and the acoustic startle response

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Abstract

Recently, the neuropeptide S (NPS) neurotransmitter system has been identified as a promising psychopharmacological drug target given that NPS has shown anxiolytic-like and stress-reducing properties and memory-enhancing effects in rodent models. NPS binds to the G-protein-coupled receptor encoded by the neuropeptide S receptor gene (*NPSR1*). A functional variant within this gene leads to an amino-acid exchange (rs324981, Asn¹⁰⁷Ile) resulting in a gain-of-function in the Ile¹⁰⁷ variant which was recently associated with panic disorder in two independent studies. A potential psychopharmacological effect of NPS on schizophrenia psychopathology was demonstrated by showing that NPS can block NMDA antagonist-induced deficits in prepulse inhibition. We therefore explored a potential role of the *NPSR1* Asn¹⁰⁷Ile variation in schizophrenia. A case-control sample of 778 schizophrenia patients and 713 healthy control subjects was successfully genotyped for *NPSR1* Asn¹⁰⁷Ile. Verbal declarative memory and acoustic startle response were measured in subsamples of the schizophrenia patients. The case-control comparison revealed that the low-functioning *NPSR1* Asn¹⁰⁷ variant was significantly associated with schizophrenia (OR 1.19, $p=0.017$). Moreover, specifically decreased verbal memory consolidation was found in homozygous Asn¹⁰⁷ carriers while memory acquisition was unaffected by *NPSR1* genotype. The schizophrenia patients carrying the Ile¹⁰⁷ variant demonstrated significantly reduced startle amplitudes but unaffected prepulse inhibition and habituation. The present study confirms findings from rodent models demonstrating an effect of NPS on memory consolidation and startle response in schizophrenia patients. Based on these findings, we consider NPS as a promising target for antipsychotic drug development.

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Key words: Acoustic startle, antipsychotic drugs, memory, neuropeptide S, schizophrenia.

Introduction

Schizophrenia is a neuropsychiatric disorder with abnormalities in various neurotransmitter systems and important contributions of genetic factors in its

pathogenesis. From the efficacy of first-generation antipsychotics due to dopamine D₂ receptor antagonism a major role of dopamine in the pathogenesis of schizophrenia was derived (Carlsson, 1978; Howes & Kapur, 2009). The development of atypical antipsychotics with lower D₂ receptor affinity such as clozapine demonstrating comparable effects of symptomatic relief in schizophrenia pointed to an additional involvement of other neurotransmitter systems (Arnt & Skarsfeldt, 1998). Translating this fundamental finding

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to the field of pharmacogenetic research gave rise to the discovery of several promising candidate genes related to other transmitter systems (reviewed in Arranz & de Leon, 2007). In return, replicable schizophrenia candidate genes modulating neurotransmission may also help to identify promising targets for future antipsychotic drug development.

Recently, neuropeptide S (NPS) has been described which binds to a G-protein-coupled receptor, the neuropeptide S receptor (NPSR) (Reinscheid & Xu, 2005b; Xu et al. 2004). In rats, NPSR mRNA is strongly expressed in several discrete nuclei or regions within the CNS including the amygdala, hypothalamus, motor cortex 2, and retrosplenial agranular cortex (Xu et al. 2004). Lower expression was found in the hippocampus and somatosensory cortex. Activation of NPSR leads to an increase of intracellular Ca^{2+} and cAMP thus indicating an excitatory effect of NPS (Reinscheid & Xu, 2005a). In contrast to NPSR which is distributed across several brain regions, precursors of NPS are only expressed in some brainstem areas including a novel locus between the noradrenergic locus coeruleus and Barrington's nucleus (Xu et al. 2004). Central administration of NPS has been shown to increase locomotor activation and to suppress sleep in rodents (Reinscheid, 2008). Moreover, mice show more exploratory behaviour in different standardized behavioural tests after injections of NPS thus suggesting a strong anxiolytic-like effect (Leonard et al. 2008; Rizzi et al. 2008; Xu et al. 2004). Recently, Fendt et al. (2010) also demonstrated the anxiety-reducing effect of NPS injections into the amygdala on the acoustic startle response. In their experiment, mice injected with 1 nmol NPS did not show the typically observed potentiation of fear when an additional shock was administered during the acoustic startle trials.

One mechanism by which NPS induces the replicable anxiolytic effect may be that it alters memory-related processes. Injections of NPS in the amygdala led to accelerated extinction of conditioned fear in mice while NPS treatment before fear conditioning did not show any effect on anxiety-related memory (Jüngling et al. 2008). Okamura et al. (2010a) extended these findings in a series of experiments yielding a specific improvement of memory consolidation with unaffected acquisition due to NPS injections. Of note, the authors also demonstrated this consolidation-promoting effect of NPS in a non-affective memory task (object recognition). Thus, it was suggested that NPS stimulates neuronal plasticity with a general enhancing effect on memory consolidation irrespective of content. In sum, NPS demonstrates anxiolytic-like

and stress-reducing effects and appears to enhance memory consolidation in animals.

NPSR is encoded by a gene located on chromosome 7p14 comprising at least nine exons (*NPSR1*). An Asn to Ile exchange at position 107 (Asn¹⁰⁷Ile, rs324981) has been identified with increased agonist potency in the Ile¹⁰⁷ variant (Reinscheid, 2008). Thus, Ile¹⁰⁷ encoded by the T-allele demonstrates a 10-fold higher NPS transmission compared to the Asn encoding A-allele (Bernier et al. 2006; Reinscheid et al. 2005b). The AA genotype of this coding variant was found to be under-represented in male patients with panic disorder while no association with schizophrenia or attention deficit hyperactivity disorder emerged (Okamura et al. 2007). This association with panic disorder was recently replicated in two independent samples by Domschke et al. (2010), who, in contrast to Okamura et al. (2007) found a stronger association in female patients. Moreover, in the Domschke et al. study, panic patients carrying the disease-related Ile¹⁰⁷ variant showed more self-reported anxiety and higher increases in heart rate during behavioural testing. Combining genotype and imaging data yielded that carriers of the Ile¹⁰⁷ variant significantly failed to recruit the dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) during the perception of fearful faces (Domschke et al. 2010).

Recently, NPS was discussed as a potential pharmacological drug target for schizophrenia (Okamura et al. 2010b). These authors investigated sensorimotor gating of the acoustic startle response in mice. Injections of MK-801, an NMDA antagonist, served as a pharmacological model of schizophrenia typically leading to disrupted prepulse inhibition (PPI). Additional injections of NPS significantly attenuated this effect as indicated by recovered PPI while the startle amplitude was not changed. Thus, by showing that NPS blocks MK-810 NMDA antagonism, this study suggests a potential antipsychotic effect of NPS (Okamura et al. 2010b). Deficient PPI is a highly replicable feature of schizophrenia across studies with some studies also reporting impaired acoustic startle reflexes (Braff et al. 2001; Hammer et al. 2011; Meincke et al. 2004; Moriwaki et al. 2009; Quednow et al. 2006b, 2008). We therefore aimed to explore whether the *NPSR1* genotype would affect sensorimotor gating and startle reflexes in patients suffering from schizophrenia.

The purpose of the present study was 2-fold: we sought (1) to examine the potential association of *NPSR1* genotype with schizophrenia in a large case-control sample and (2) to elucidate the functional

characteristics of the *NPSR1* Asn¹⁰⁷Ile variation on neurocognition in schizophrenia. Based on the findings of Okamura *et al.* (2007, 2010b) we therefore analysed sensorimotor gating applying an acoustic startle paradigm and verbal declarative memory. It was hypothesized that the *NPSR1* genotype would affect the acoustic startle amplitude, PPI, and memory consolidation but not memory acquisition.

Experimental procedures

Sample

Schizophrenia patients ($n=778$) were recruited from the Departments of Psychiatry of the Universities of Bonn, Düsseldorf, Cologne, and from the Central Institute of Mental Health of Mannheim, Germany. Diagnoses were established according to the criteria of ICD-10 (WHO, 1992) and DSM-IV (APA, 1994) by experienced psychiatrists or clinical psychologists. Healthy control subjects ($n=713$) were recruited from community registers or via local advertisement and were screened for the absence of any psychiatric disorder. The study was approved by the appropriate ethics committees and all subjects gave written informed consent prior to inclusion. Mean age of schizophrenia patients was 34.1 yr (s.d.=10.6 yr) while healthy control subjects were 39.3 yr (s.d.=13.5 yr) on average. Four hundred and sixty (59.1%) schizophrenia patients and 338 (48.3%) healthy controls were male.

Verbal declarative memory was tested in a subset of 199 schizophrenia patients and 204 control subjects allowing us to explore the functional consequences of *NPSR1* genotype. In the neuropsychologically characterized sample, neither age, sex distribution, and pre-morbid education as assessed with the MWT-B in the schizophrenia patients and the healthy control subjects, nor age at onset and duration of illness in the schizophrenia patients were related to *NPSR1* genotype (all $p>0.179$). In the healthy volunteers, recognition data were only available for 175 subjects.

Acoustic startle response was measured in a second subset of 71 schizophrenia patients of which 26 were included in the neuropsychological sample. All of these subjects were inpatients admitted to the Department of Psychiatry and Psychotherapy of the University of Bonn and were considered eligible for the study if the following criteria were met: a diagnosis of schizophrenia according to DSM-IV, age between 18 and 65 years, and central European ancestry. Patients were excluded if they had a history of a neurological disease, substance dependency, or a

severe somatic disease. In this subset, every patient was evaluated by a Structured Clinical Interview (SCID-I) according to DSM-IV and clinical symptoms were measured with the Positive and Negative Syndrome Scale (PANSS; Kay *et al.* 1992). In this sample, the *NPSR1* genotype was also unrelated to age, sex, education, medication status and dose, and PANSS symptom rating (all $p>0.10$).

Measures

Neuropsychological measures included the German version of the Auditory Verbal Learning Test (AVLT, Helmstaedter *et al.* 2001). The AVLT is a standardized measure of verbal declarative memory asking the subjects to learn and recall 15 items of a word list. Selected outcome variables were (1) recall after the first trial, (2) total recall over trials 1–5, (3) recall after interference (trial 6), (4) delayed recall after 30 min (trial 7), and (5) recognition as indicated words correctly recognized [p(A) according to Geffen *et al.* (1990)]. In addition, pre-morbid verbal education was assessed with the Mehrfachwahl-Wortschatz-Test (MWT-B), a German vocabulary test (Lehrl *et al.* 1999).

PPI was recorded and analysed as described in our previous work (Quednow *et al.* 2006a,b, 2008). Acoustic startle stimuli were presented binaurally through headphones (TDH-39-P; Maico). Each session began with a 4-min acclimation period of 70 dB background white noise that was continued throughout the session. Participants received 73 sound pulses with a power of 116 dB along with 70 dB background white noise. In 36 of the trials, the pulse was preceded by an 86-dB prepulse with an inter-stimulus interval (ISI) of 120 ms. The eye-blink component of the acoustic startle response (right eye) was measured using an electromyographic startle system (San Diego Instruments, USA). Recorded EMG activity was bandpass-filtered (1–1000 Hz). A 50-Hz notch filter was also used to eliminate 50-Hz interference. The amplifier gain was kept constant for all participants, and the EMG was recorded from the onset of the acoustic startle stimulus for 250 ms with a sampling rate of 1 ms.

Voluntary and spontaneous blinks were excluded from further analysis using the registration parameters described by Braff *et al.* (1992). The latency to startle response onset was defined by a shift of 2.28 mV (six digital units) from the baseline value occurring 21–120 ms after the acoustic startle stimulus. Latency-to-response peak was defined as the point of maximal amplitude that occurred within 150 ms after

the startle stimulus. Response rejections were made both in case of onset-to-peak latencies >95 ms and baseline shifts >34.2 mV (>90 digital units). Two patients (2.8% of the total sample) with more than 50% response rejections were excluded from data analysis. All remaining subjects show a startle amplitude >25 units – the commonly used threshold for analysable startle data (Braff *et al.* 1992). Smoking *ad libitum* was permitted before testing (Kumari & Gray, 1999).

Genotyping

DNA for single nucleotide polymorphism (SNP) genotyping was isolated from EDTA anticoagulated blood using the Qiagen protocol for Blood & Cell Culture DNA Maxi kit (Qiagen, Germany). PCR was performed using 12.5 ng DNA, the Taqman[®] Universal PCR MasterMix, No AmpErase[®] UNG and the Taqman[®] SNP Genotyping Assays for each SNP (all provided by Applied Biosystems, USA) according to the protocol for Taqman[®] SNP genotyping (Applied Biosystems). Each assay consisted of the unlabelled forward and reverse primers and the FAM and VIC dye-labelled MGB probes. These assays are designed for allelic discrimination of specific SNPs. Both alleles were scored in a single well by measuring the fluorescence at the end of PCR using a Tecan Ultra 384 reader (Tecan, Germany). Excitation- and emission-wavelengths for the FAM-labelled probes were 485 and 535 nm and for the VIC-labelled probes 535 and 590 nm, respectively.

Statistics

Armitage trend test was employed to analyse case-control data using the FamHap program (Becker & Knapp, 2004). The mean %PPI of startle amplitude was calculated using the formula:

$$\% \text{ PPI} = 100 \times \frac{\text{amplitude on PA trials} - \text{amplitude on PP trials}}{\text{amplitude on PA trials}},$$

where PA = pulse-alone and PP = prepulse (Braff *et al.* 1992). For the assessment of startle habituation, PA trials were each divided into six blocks. The % habituation was calculated as the reduction in startle magnitude between the first and last block of PA and PP trials:

$$\% \text{ habituation} = 100 \times \frac{\text{first block} - \text{last block}}{\text{first block}}$$

(Geyer & Braff, 1982). As a further measure for habituation, the linear gradient coefficient b was

calculated across six blocks of PA and PP trials within each subject

$$b = \left(n \sum xy - \left(\sum x \right) \left(\sum y \right) \right) / \left(n \sum x^2 - \left(\sum x \right)^2 \right),$$

where x = block number, y = startle amplitude PA trial per block, according to Geyer & Braff (1982). Startle reactivity was assessed by the mean amplitude of the first block of PA trials and the mean amplitude of all PA trials. All data were analysed by analysis of variance (ANOVA) with exception of frequency data. Frequency data were analysed using χ^2 tests. The confirmatory statistical comparisons were performed with significance level set at $p < 0.05$ (two-tailed).

Results

First, we compared *NPSR1* genotype distribution in our large case-control sample comprising 778 schizophrenia patients and 713 healthy control subjects. The Asn¹⁰⁷Ile polymorphism of the *NPSR1* gene was in Hardy–Weinberg equilibrium in the schizophrenia patients and healthy control subjects ($p = 0.720$ and 0.823 , respectively). Homozygosity for the Asn¹⁰⁷ variant was found in 31.2% of schizophrenia patients and in 26.6% of healthy control subjects. Conversely, homozygosity for the Ile¹⁰⁷ variant was found in only 18.9% of schizophrenia patients and 23.0% of healthy comparison subjects. Thus schizophrenia patients were more likely to carry the low functioning Asn¹⁰⁷ variant compared to the healthy control group [Armitage trend test: $\chi^2(1) = 5.711$, $p = 0.01685$; odds ratio: Asn¹⁰⁷ = 1.19, 95% CI 1.03–1.38]. Complete genotype data of the case-control sample is given in Table 1.

We analysed verbal declarative memory in the schizophrenia patients and healthy control subjects computing a series of 2×2 ANOVAs. Given that the low-functioning Asn¹⁰⁷ variant was significantly related to schizophrenia in this study and based on previous studies (Domschke *et al.* 2010; Okamura *et al.* 2007), we statistically compared homozygous Asn¹⁰⁷ subjects (Asn/Asn) against carriers of the Ile¹⁰⁷ variant (Ile).

As expected, all ANOVAs showed marked memory impairments in the schizophrenia patients compared to the healthy control group (all $p < 0.001$). Following the AVLt test protocol, no significant genotype effects or genotype \times diagnosis interactions were found for the first trial (genotype: $F_{1,399} = 0.24$, $p = 0.624$; interaction: $F_{1,399} = 0.49$, $p = 0.480$) or with regard to total learning over all five trials (genotype: $F_{1,399} = 1.12$, $p = 0.290$; interaction: $F_{1,399} = 1.13$, $p = 0.288$). Analysing

Table 1. Association of the Asn¹⁰⁷Ile polymorphism of the neuropeptide S receptor (*NPSR1*) gene in a large schizophrenia case-control sample

	Schizophrenia patients (<i>n</i> = 778)	Healthy controls (<i>n</i> = 713)	OR (95 % CI)
<i>NPSR1</i> genotype ^a			
Asn/Asn (AA)	243 (31.2 %)	190 (26.6 %)	1.25 (0.99–1.56)
Asn/Ile (AT)	388 (49.9 %)	359 (50.4 %)	0.98 (0.80–1.20)
Ile/Ile (TT)	147 (18.9 %)	164 (23.0 %)	0.78 (0.61–1.00)
<i>NPSR1</i> alleles ^b			
Asn (A allele)	874 (56.2 %)	739 (51.8 %)	1.19 (1.03–1.38)
Ile (T allele)	682 (43.8 %)	687 (48.2 %)	0.84 (0.73–0.97)

OR, Odds ratio; CI, confidence interval.

^a χ^2 test on genotype distribution: $p = 0.057$.

^b Armitage trend test: global $p = 0.017$.

Table 2. Verbal memory in schizophrenia patients and healthy controls stratified for Asn¹⁰⁷Ile of the *NPSR1* gene

	Schizophrenia patients				Healthy controls			
	Asn/Asn	Asn/Ile	Ile/Ile	Asn/Asn vs. Ile	Asn/Asn	Asn/Ile	Ile/Ile	Asn/Asn vs. Ile
<i>N</i>	59	105	35		50	105	49	
AVLT trial 1	6.3 (2.1)	6.6 (1.8)	6.8 (1.6)	$p = 0.539$	7.9 (2.4)	7.8 (2.2)	7.9 (1.9)	$p = 0.934$
AVLT trials 1–5	47.6 (11.7)	49.9 (10.6)	50.2 (12.3)	$p = 0.169$	57.7 (8.3)	57.5 (9.3)	58.1 (8.1)	$p = 0.997$
AVLT trial 6	9.0 (3.3)	10.0 (3.1)	10.0 (4.2)	$p = 0.047$	11.9 (2.5)	12.0 (2.7)	12.8 (2.3)	$p = 0.361$
AVLT trial 7	9.0 (3.6)	10.0 (3.1)	10.2 (3.9)	$p = 0.032$	12.1 (2.4)	12.3 (2.6)	12.8 (2.5)	$p = 0.446$
AVLT recognition p(A)	0.74 (0.16)	0.80 (0.16)	0.78 (0.15)	$p = 0.034$	0.89 (0.13)	0.88 (0.15)	0.89 (0.15)	$p = 0.716$

AVLT, Rey auditory verbal learning test.

Data are expressed as mean with standard deviation (S.D.) in parentheses. Bold numbers indicate significant findings.

AVLT free recall after interference (trial 6) showed a significant main effect for genotype with an insignificant interaction (genotype: $F_{1,399} = 4.49$, $p = 0.035$; interaction: $F_{1,399} = 0.94$, $p = 0.332$) indicating impairments in homozygous Asn¹⁰⁷ carriers compared to Ile¹⁰⁷ subjects (Asn/Asn = 10.3 ± 3.3 , Ile = 11.2 ± 3.2). The same pattern was found for AVLT delayed recall (trial 7): subjects with the Asn/Asn genotype recalled fewer words after 30 min compared to subjects with at least one Ile¹⁰⁷ allele (Asn/Asn = 10.4 ± 3.5 , Ile = 11.3 ± 3.2 ; genotype: $F_{1,399} = 4.66$, $p = 0.032$; interaction: $F_{1,399} = 1.50$, $p = 0.222$). With regard to recognition, an insignificant main effect for genotype and a genotype \times diagnosis interaction effect on trend-level was found (genotype: $F_{1,370} = 1.43$, $p = 0.233$; interaction: $F_{1,370} = 2.95$, $p = 0.087$). Inspection of the means indicated a specifically reduced recognition in Asn/Asn schizophrenia patients compared to Ile¹⁰⁷ patients while no

such effect was evident in the healthy control subjects (Table 2).

In a second step, we also analysed the effects of the *NPSR* genotype in the schizophrenia patients and the healthy control group separately to further explore which group contributed to the effects reported above. The schizophrenia patients with the Asn/Asn variant and patients with an Ile¹⁰⁷ variant showed comparable memory acquisition during the first trial and with regard to total learning over all five trials ($p > 0.10$). However, when analysing free recall of the word list, significant genotype effects emerged in schizophrenia patients. Thus, patients homozygous for the Asn¹⁰⁷ variant recalled fewer words after interference ($F_{1,197} = 3.99$, $p = 0.047$) and after delay ($F_{1,197} = 4.64$, $p = 0.032$) compared to patients with the Ile¹⁰⁷ variant. This genotype effect was also found with regard to recognition with a reduced probability of correct

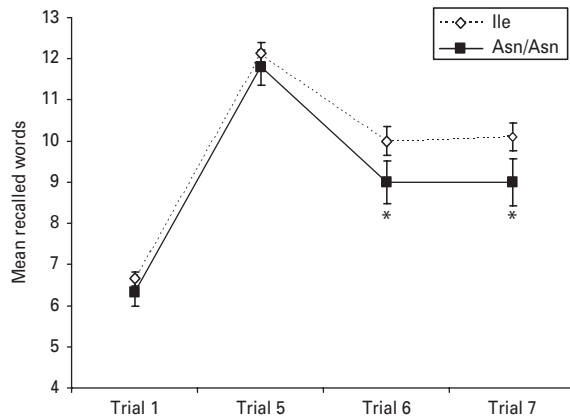


Fig. 1. Specifically impaired memory consolidation in schizophrenia patients homozygous for the *NPSR1* Asn¹⁰⁷ variant. Ile, Carriers of the Ile variant (Asn/Ile or Ile/Ile). Mean words retrieved are given with standard error of the mean (S.E.M.). Trials 1–5 are acquisition trials, trials 6 and 7 are free recall trials after an interference list and after delay. * $p < 0.05$.

recognition [p(A)] in Asn/Asn carriers compared to Ile¹⁰⁷ subjects ($F_{1,197} = 4.53$, $p = 0.034$). These results suggest a specific effect of *NPSR1* genotype on memory consolidation in schizophrenia with free recollection and recognition being affected while acquisition remains intact (see Fig. 1 for illustration). In the healthy control subjects, no effect of *NPSR1* genotype on memory performance was found ($p > 0.20$). Complete verbal declarative memory data of schizophrenia patients and healthy controls is given in Table 2.

In the schizophrenia subpopulation assessed with the acoustic startle paradigm, the Asn¹⁰⁷Ile polymorphism was distributed in accordance to Hardy–Weinberg equilibrium and *NPSR1* genotype groups did not differ regarding demographic and clinical data (see Table 3).

The means (\pm S.E.M.) of the acoustic startle paradigm are given in Table 3. In contrast to our expectations based on the study of Okamura *et al.* (2010b), PPI was not significantly modulated by the coding *NPSR1* polymorphism Asn¹⁰⁷Ile ($p > 0.20$). However, both startle reactivity measures (mean amplitude of PA trials in the first block as well as across all blocks) were consistently affected by genotype with carriers of the Ile¹⁰⁷ variant showing significantly reduced startle amplitudes compared to patients homozygous for the Asn¹⁰⁷ variant (first block of PA trials: $t_{67} = 2.7$, $p = 0.008$; all PA trials: $t_{67} = 3.2$, $p = 0.003$) (Fig. 2).

To investigate the impact of possible confounding factors we also introduced age, gender, medication status, smoking, and education as separate covariates in ANCOVAs of the genotype effect on startle

reactivity. The *NPSR1* genotype effect on both startle reactivity measures remained significant in every analysis (at least $p < 0.05$). Age was the only significant covariate (first block of PA trials: $p = 0.004$; all PA trials: $p = 0.003$) and correlation analysis showed that startle reactivity decreases with age (both startle parameters: $r = -0.44$, $p < 0.001$).

Habituation parameters were not significantly influenced by *NPSR1* rs324981 genotype (see Table 3).

Additionally, we compared schizophrenia patients of the present study with 46 matched (according to age, gender, and education) but non-genotyped healthy control subjects, who were assessed with the same PPI paradigm in our previous studies (Quednow *et al.* 2006b, 2008). The total group of schizophrenia patients displayed significantly decreased PPI (mean \pm S.E.M. %PPI in controls: 56.9 ± 3.2 , $t_{113} = 3.1$, $p = 0.002$) and diminished startle reactivity in the first block of PA trials but not across all PA trials (first block of PA trials in controls: 382 ± 45.1 arbitrary units, $t_{113} = 2.6$, $p = 0.01$; all PA trials in controls 241 ± 29.3 arbitrary units, $t_{113} = 1.3$, $p > 0.20$) compared to controls. To examine the direction of the genotype effect on startle reactivity, we compared healthy controls with both genotype groups (Asn/Asn *vs.* Asn/Ile + Ile/Ile) of schizophrenia patients. Patients carrying the Ile¹⁰⁷ variant showed significantly reduced startle amplitudes compared to healthy controls (first block of PA trials: $t_{97} = 3.2$, $p = 0.002$; all PA trials: $t_{97} = 2.1$, $p = 0.04$), whereas patients homozygous for the Asn¹⁰⁷ variant did not differ from the normal population (first block of PA trials: $t_{60} = 0.2$, $p > 0.80$; all PA trials: $t_{60} = 1.1$, $p > 0.25$).

Discussion

The present study investigated the functional polymorphism Asn¹⁰⁷Ile of the neuropeptide S receptor gene (*NPSR1*) in schizophrenia. In a large case-control sample, the schizophrenia patients were more likely to carry the low-functioning Asn¹⁰⁷ variant compared to the healthy control group. Moreover, compared to carriers of the Ile variant, subjects homozygous for the Asn¹⁰⁷ variant were significantly impaired in verbal memory functioning in a neuropsychologically characterized subsample. This finding was also observed in the sample of schizophrenia patients but was not seen in healthy control subjects alone. While PPI was not affected by *NPSR1* genotype, strongly decreased startle reactivity was found in the schizophrenia patients with the Ile¹⁰⁷ variant.

According to a study conducted by Reinscheid *et al.* (2005b), the Ile¹⁰⁷ variant encoded by the T-allele

Table 3. Demographic data and psycho-physiological parameters of 69 schizophrenia patients grouped according to their *NPSR1* Asn¹⁰⁷Ile genotype

<i>NPSR1</i> Asn ¹⁰⁷ Ile variant	Asn/Asn	Asn/Ile	Ile/Ile	Total	F/χ^2	df/df_{err}	p	η_p^2
N^a	16 (23.2%)	40 (58%)	13 (18.8%)	69 (100%)	1.43	1	0.23	–
Age (yr)	31.9 (2.4)	35.8 (1.7)	34.8 (3.5)	34.7 (1.3)	0.73	2/66	0.49	0.02
Years of education	14.7 (0.8)	14.0 (0.5)	15.9 (0.5)	14.5 (0.4)	1.82	2/66	0.17	0.06
Men (%)	75.0%	67.5%	69.2%	69.6%	0.30	2	0.86	–
Current smokers (%)	50.0%	55.0%	53.8%	53.6%	0.11	2	0.94	–
Medication status (%) (unmedicated/typical/ atypical antipsychotic)	31.3/25.0/43.8%	15.0/15.0/70%	23.1/15.4/61.5%	20.3/17.4/62.3%	3.51	4	0.48	–
Daily chlorpromazine equivalents ^b	203 (58.2)	297 (33.3)	221 (53.2)	261 (25.9)	1.41	2/66	0.25	0.05
PANSS positive ^c	17.1 (1.9)	19.3 (1.2)	16.4 (2.5)	18.2 (0.9)	0.52	1/68	0.47	0.01
PANSS negative ^c	19.8 (1.6)	20.8 (1.2)	20.5 (2.4)	20.5 (0.9)	0.19	1/68	0.66	0.00
PANSS general ^c	39.1 (3.1)	41.2 (2.3)	36.9 (3.8)	40.0 (1.7)	0.09	1/68	0.77	0.02
PANSS total ^c	76.0 (5.9)	82.2 (4.2)	73.8 (7.9)	79.3 (3.1)	0.35	1/68	0.56	0.01
First block, mean amplitude of pulse-alone trials (arbitrary units) ^c	363 (50.5)	241 (27.1)	201 (43.6)	262 (22.1)	6.96	1/68	0.01	0.09
Mean amplitude of all pulse-alone trials (arbitrary units) ^c	304 (41.7)	176 (24.8)	141 (34.2)	199 (19.6)	9.79	1/68	0.003	0.13
Mean percent prepulse inhibition (SOA 120 ms) ^c	40.7% (6.8)	44.4% (4.4)	35.6% (7.8)	41.9% (3.3)	0.04	1/68	0.84	0.00
Percent early habituation of pulse-alone trials (between first and second blocks) ^c	18.1% (5.9)	27.9% (4.3)	28.8% (7.2)	25.8% (3.1)	1.86	1/68	0.18	0.03
Percent total habituation of pulse-alone trials (between first and sixth blocks) ^c	29.3% (6.9)	42.9% (5.1)	48.6% (7.7)	40.8% (3.7)	3.01	1/68	0.09	0.05
Habituation of pulse-alone trials across six blocks (linear gradient coefficient b) ^c	–17.8 (5.9)	–16.8 (2.6)	–15.2 (1.9)	–16.7 (2.2)	0.07	1/68	0.79	0.00

PANSS, Positive and Negative Syndrome Scale; SOA, stimulus onset asynchrony.

Means are given with standard error of means (S.E.M.) in parentheses. Gender and smoking status are expressed in frequency data.

^a Fisher's exact test on violation of the Hardy–Weinberg equilibrium.

^b Unmedicated patients received the value zero.

^c Statistically comparing Asn/Asn *vs.* Ile carriers.

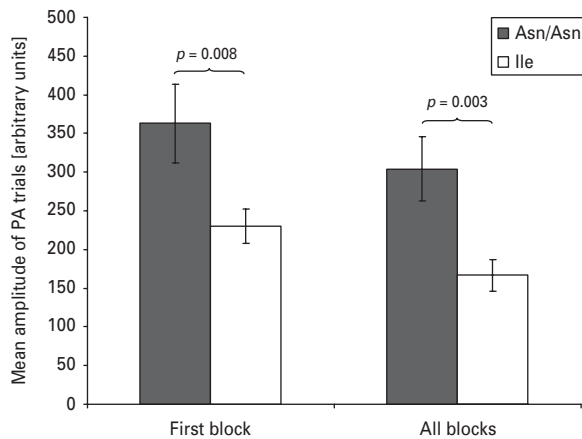


Fig. 2. Significantly reduced startle amplitude in schizophrenia patients carrying the *NPSR1* Ile¹⁰⁷ variant. Ile, Carriers of the Ile variant (Asn/Ile or Ile/Ile). Mean startle amplitudes of pulse-alone (PA) trials are given and bars indicate standard error of the mean (S.E.M.). *t* tests were performed for statistical comparisons.

demonstrates increased agonist potency by a factor of 10. The higher frequencies of the low-functioning Asn¹⁰⁷ variant in schizophrenia patients compared to healthy control subjects therefore suggest a genetically driven deficiency in the NPS system in schizophrenia. The association with schizophrenia reported here is in contrast to Okamura *et al.* (2007) who did not find any association for *NPSR1* genotype and schizophrenia. However, the study conducted by Okamura *et al.* included only 221 schizophrenia patients and 245 healthy control subjects of whom the male control subjects were not in Hardy–Weinberg equilibrium. In contrast our sample consisted of nearly 1500 subjects resulting in a 3-fold larger sample. This suggests that Okamura and colleagues might not have had sufficient statistical power to detect the association of *NPSR1* genotype with schizophrenia reported here. While our study suggests a role of the Asn¹⁰⁷ variant in schizophrenia, two independent studies confirmed higher frequencies of the Ile¹⁰⁷ variant in patients with panic disorder (Domschke *et al.* 2010; Okamura *et al.* 2007). Although the latter association was somewhat unexpected with regard to findings from rodent models demonstrating strong anxiolytic effects of NPS, Domschke and colleagues suggest that the *NPSR1*-related risk for panic disorder might be conferred by an arousal-promoting effect of the Ile¹⁰⁷ variant. The disease-specific pattern of the *NPSR1* genotype association with schizophrenia and panic disorder suggests that NPS may exert a differential effect within the development of these two disorders.

Meta-analytical studies on neuropsychological functions in schizophrenia patients document large impairments of up to 1.5 s.d. units in verbal memory (Aleman *et al.* 1999; Heinrichs & Zakzanis, 1998). In our study, the schizophrenia-associated *NPSR1* variant Asn¹⁰⁷ modulated specific verbal memory functions. Homozygous carriers of this variant showed significantly impaired retrieval and recognition of a word list compared to subjects with the high-functioning Ile¹⁰⁷ variant while memory acquisition was comparable between the different genotypes. A recent experimental animal trial conducted by Okamura *et al.* (2010a) directly investigated NPS effects on memory. The authors observed increased step-trough latencies in an inhibitory avoidance paradigm after NPS injections thus confirming earlier findings of NPS facilitating fear-related memory (Jüngling *et al.* 2008; Okamura *et al.* 2010a). Moreover, NPS also enhanced memory and recognition of non-affective objects suggesting a more general effect of NPS on memory processing. Importantly, the memory-enhancing effect of NPS was only seen when the injections were administered before the test phase while injections before the training phase showed no effect. Based on this finding, a specific effect of NPS on memory consolidation with unaffected acquisition was derived (Okamura *et al.* 2010a). Verbal memory tested in human subjects, as employed in the present study, and long-term object recognition tested in rodents, as investigated in the Okamura *et al.* study, are not directly comparable. However, it is interesting that both studies suggest a specific effect of NPS availability on memory consolidation.

The exact pharmacological properties of NPS are not yet fully understood. Examinations of the behavioural phenotype of *NPSR1* knock-out mice have yielded inconsistent findings with regard to anxiety. While Duangdao *et al.* (2009) found increased anxiety behaviour in *NPSR* knock-out mice in the open field, the elevated maze, and the light-dark box compared to wild-type animals, no effect on startle response or PPI was evident. Fendt *et al.* (2011) also reported *NPSR* knock-out mice to be more anxious in the elevated plus maze but showing a significantly decreased acoustic startle amplitude compared to their wild-type littermates. However, injections of NPS reliably induce an anxiolytic-like effect in animals (Leonard *et al.* 2008; Rizzi *et al.* 2008; Xu *et al.* 2004). For instance Leonard *et al.* (2008) showed comparable behavioural effects of NPS and the benzodiazepines alprazolam and chlordiazepoxide in mice using different tasks thought to measure anxiety. These authors concluded that NPS may be a novel therapeutic target for the

treatment of anxiety disorders. Fendt *et al.* (2010) also investigated the effect of NPS on mice in a fear-potentiated acoustic startle paradigm. Usually, in this task the application of an additional electric shock leads to a considerable increase of the startle amplitude. However, in the study conducted by Fendt *et al.* (2010) this effect could be successfully blocked by injection of 1 nmol NPS in the amygdala.

Recently, Okamura *et al.* (2010b) also provided evidence for a potential antipsychotic effect of NPS. They tested a pharmacological model of psychosis in mice using MK-801, an NMDA antagonist, which elicits strong impairments in sensorimotor gating as indicated by disrupted PPI. Typically this effect can be blocked by atypical antipsychotic agents such as clozapine (Geyer & Ellenbroek, 2003). Pre-treatment with NPS essentially abrogated the MK-801 effect on PPI (Okamura *et al.* 2010b). In the present study, analyses of acoustic startle responses yielded mixed results. On the one hand, we did not observe any effect of *NPSR1* genotype on PPI, while on the other hand, startle reactivity was decreased in carriers of the high-functioning Ile¹⁰⁷ variant in our study, which confirms earlier findings of an anxiolytic effect of NPS on startle reactivity and other anxiety-related phenotypes (Fendt *et al.* 2010; Jüngling *et al.* 2008; Leonard *et al.* 2008; Reinscheid *et al.* 2005a; Rizzi *et al.* 2008; Xu *et al.* 2004). Thus, carriers of the Ile¹⁰⁷ variant determining a gain of NPS functionality may exhibit lowered arousal, which subsequently leads to significantly reduced startle reflexes. Surprisingly, the Asn¹⁰⁷ variant with higher prevalence in schizophrenia patients was associated with normal startle amplitudes, although we and others have shown that schizophrenia patients display lowered startle reactivity, as was additionally confirmed in comparison to a healthy control group in the present study (Braff *et al.* 2001; Hammer *et al.* 2011; Meincke *et al.* 2004; Moriwaki *et al.* 2009; Quednow *et al.* 2006b, 2008). Thus, the risk allele for schizophrenia appears to be protective against psychosis-related hyporeactivity regarding the acoustic startle response. Consequently, the *NPSR1* Ile¹⁰⁷ variant might interact with another gene to cause both lowered startle response and forms of schizophrenia in which *NPSR1* plays a less significant role. Moreover, the Ile¹⁰⁷ variant has been shown to be associated with panic disorder (Domschke *et al.* 2010; Okamura *et al.* 2007), in which higher autonomic arousal is evident. However, whether patients with panic disorder also show an elevated startle response in general is not clear so far. Many studies failed to demonstrate increased startle responses to acoustic stimuli in panic disorder under neutral conditions, in which no fearful

stimuli were presented and no fearful imagery was demanded (Amrhein *et al.* 2005; Favaron *et al.* 2010; Grillon *et al.* 1994; McTeague *et al.* 2011). Thus, our results are not necessarily in contrast to previous literature on the psychophysiology of panic disorder. Unfortunately, we do not have genotype data from healthy controls and were therefore unable to test for a possible startle and anxiety-modulating effect of the *NPSR1* Asn¹⁰⁷Ile polymorphism in psychiatrically unaffected subjects.

In conclusion, the schizophrenia patients in our study had a reduced prevalence of the high-functioning *NPSR1* Ile¹⁰⁷ variant probably leading to a less active NPS system and contributing to the memory impairments typically observed in these patients. An interaction of the *NPSR1* gene and other schizophrenia susceptibility genes may contribute to a differentially modulated startle response. We suggest that adjuvant administration of NPS might show antipsychotic effects in patients with schizophrenia and might help to improve cognitive disruptions like impaired memory functions in schizophrenia.

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Statement of Interest

None.

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